FLOW CYTOMETRY SHARED RESOURCE

STANDARD OPERATING PROCEDURE

BD LSRFortessa-HTS – Operation, Calibration and Maintenance

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Approved By:

Director of Flow Cytometry Shared Resource	**DATE**
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1.0	Development of SOP	03-07-2017	pguevara	Dr. Gray
2.0	Update facility name, instrument workstation and operating procedures	07-24-2019	pguevara	Dr. Gray
3.0	Update software versions, minor language changes	02-01-2022	pguevara	Dr. Wieder
3.1	Review	09-25-2022	pguevara	Dr. Wieder

PURPOSE

The purpose of this standard operating procedure is to describe the procedures used by the University of Miami, Miller School of Medicine Sylvester Comprehensive Cancer Center Flow Cytometry Shared Resource staff in the operation, maintenance, and calibration of the BD LSRFortessa-HTS as well as general user operation of the instrument.

SCOPE

This standard operating procedure applies to the use of the BD LSRFortessa-HTS flow cytometer by SCCC Flow Cytometry Shared Resource personnel and users of the Flow Cytometry Shared Resource.

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1. AUTHORITY AND RESPONSIBILITY

1.1 The UM SCCC Flow Cytometry Shared Resource has the authority to establish these procedures and is in control of the Standard Operating Procedure (SOP) document.

1.2 The UM SCCC Flow Cytometry Shared Resource is responsible for the implementation of these procedures and ensuring staff and facility users are appropriately trained.

1.3 The users of the UM SCCC Flow Cytometry Shared Resource are responsible for understanding and following the regulations and guidelines set forth by the core facility with regards to instrument operation and biosafety procedures.

2. TARGET USERS

2.1 UM SCCC Flow Cytometry Shared Resource staff.

2.2 Internal (university members) and external (academic and commercial) users of the UM SCCC Flow Cytometry Shared Resource BD LSRFortessa-HTS.

3. DEFINITIONS

3.1 PMT: photomultiplier tube is one type of signal detector used to detect the weaker signals generated by side scatter and all fluorescence channels.

3.2 Biosafety Level: is a required set of precautions and guidelines used to isolate any potentially dangerous biological agent within a laboratory. The Centers for Disease Control have specified and defined the 4 biosafety levels.

3.3 SIP: sample injection port

3.4 BAL seal: rubber gasket which allows pressurization of the sample tube when loaded on the SIP.

3.5 CS&T: Cytometer Setup and Tracking. Beads used to define the baseline performance of all the fluorescence detectors in the cytometer. These beads are also used for daily performance checks of all the fluorescence detectors.

3.6 PM: Preventative Maintenance. Completed by the BD service engineer as part of the service contract and done every 6 months.

4. REFERENCES / MANUALS

4.1 BD LSRFortessa User’s Guide

4.2 BD High Throughput Sampler User’s Guide

4.3 BD FACSDiva Software (v.9.0.1)

4.4 BD Cytometer Setup and Tracking Application Guide

4.5 Coherent Laser System Operator’s Manual

5. INSTRUMENTATION

5.1 BD LSRFortessa
  5.1.1 Serial #: H80000006
  5.1.2 Manufacturing Date: April 2010
  5.1.3 Laser Configuration: 355nm (ULTRAVIOLET) – Trigon
    5.1.3.1 PMT “A”: 530/30 BP, 505 LP [Indo-1 (Blue)]
    5.1.3.2 PMT “B”: 450/50 BP [Indo-1 (Violet)]
    5.1.3.3 PMT “C”: EMPTY
  5.1.4 Laser Configuration: 405nm (VIOLET) - Octagon
    5.1.4.1 PMT “A”: 710/50 BP, 685 LP [Qdot 705]
    5.1.4.2 PMT “B”: 662/25 BP, 635 LP [Qdot 655]
    5.1.4.3 PMT “C”: 610/20 BP, 600 LP [Qdot 605]
    5.1.4.4 PMT “D”: 525/50 BP, 505 LP [AmCyan]
    5.1.4.5 PMT “E”: 450/50 BP [Pacific Blue]
    5.1.4.6 PMT “F”: EMPTY
    5.1.4.7 PMT “G”: EMPTY
    5.1.4.8 PMT “H”: EMPTY
  5.1.5 Laser Configuration: 488nm (BLUE) - Octagon
    5.1.5.1 PMT “A”: EMPTY
    5.1.5.2 PMT “B”: 695/40 BP, 685 LP [PerCP-Cy5.5]]
    5.1.5.3 PMT “C”: 670/30 BP, 635 LP [PerCP]
    5.1.5.4 PMT “D”: 530/30 BP, 505 LP [FITC]
    5.1.5.5 PMT “E”: 488/10 BP [SSC]
    5.1.5.6 PMT “F”: EMPTY
    5.1.5.7 PMT “G”: EMPTY
    5.1.5.8 PMT “H”: EMPTY
  5.1.6 Laser Configuration: 561nm (YELLOW-GREEN) – Octagon
    5.1.6.1 PMT “A”: 780/60 BP, 750 LP [PE-Cy7]
    5.1.6.2 PMT “B”: 710/50 BP, 690 LP [PE-Cy5.5]
    5.1.6.3 PMT “C”: 670/30 BP, 640 LP [PE-Cy5]
    5.1.6.4 PMT “D”: 610/20 BP, 600 LP [PE-Texas Red]
    5.1.6.5 PMT “E”: 582/15 BP [PE]
    5.1.6.6 PMT “F”: EMPTY
    5.1.6.7 PMT “G”: EMPTY
    5.1.6.8 PMT “H”: EMPTY
  5.1.7 Laser Configuration: 640nm (RED) - Trigon
    5.1.7.1 PMT “A”: 780/60 BP, 750 LP [APC-Cy7]
    5.1.7.2 PMT “B”: 730/45 BP, 685 LP [Alexa Fluor 700]
    5.1.7.3 PMT “C”: 670/30 [APC]
5.2 BD High Throughput Sampler
  5.2.1 Serial #: U70100115

5.2.2 Manufacturing Date: November 2010
5.2.3 Preventive Maintenance Kit (BD part# 337042). Parts sold individually if needed.
5.2.3.1 Glass syringe: BD part# 344912
5.2.3.2 Tubing assembly, secondary: BD part# 335452
5.2.3.3 Tubing assembly, primary: BD part# 335454
5.2.3.4 Tubing assembly, injection port: BD part# 335526
5.2.3.5 Probe and tubing assembly: BD part# 34389017
5.2.3.6 HTS sheath filter: BD part# 335710
5.2.3.7 Quick-disconnect O-ring: BD part# 343618
5.2.3.8 O-ring sleeve retainer: BD part# 343620
5.2.3.9 Air filter: BD part# 336218
5.2.3.10 Absorbant pad: BD part# 343502
5.2.3.11 Injection tubing to secondary pump: BD part# 335451
5.3 BD LSRFortessa-HTS Fluidics System
5.3.1 Parts not covered by service contract
5.3.1.1 Sheath Filter Assembly: BD part# 344678
5.4 BD LSRFortessa-HTS Workstation
5.4.1 HP Z240 Workstation
5.4.2 Operating System: Windows 10

6. BIOSAFETY

6.1 Regulations and Guidelines
6.1.1 BD LSRFortessa-HTS operates under Biosafety Level 1 (BSL-1) conditions ONLY.
6.1.2 Biosafety Level 2 cells cannot be processed. If BSL-2 cells need to be acquired on the BD LSRFortessa-HTS, they must be fixed and rendered non-infectious using an appropriate fixative (4% paraformaldehyde, 4% formaldehyde, or 10% formalin; alcohols are not adequate in rendering BSL-2 cells, BSL-1).
6.1.3 Biosafety Level 3 and Biosafety Level 4 cells CANNOT be processed on any instrument within the facility.
6.2 Clinical samples
6.2.1 Clinical samples CANNOT be processed in the SCCC Flow Cytometry Shared Resource.
6.2.1.1 Exception #1: Clinical samples designated “FOR RESEARCH ONLY” are acceptable.

7. MATERIALS

7.1 Other Equipment
7.1.1 Vortex

- 7.2 Reagents
  - 7.2.1 BD Cytometer Setup and Tracking beads
    - 7.2.1.1 BD Cat# 655051
  - 7.2.2 FACS Flow
    - 7.2.2.1 BD Cat# 342003
    - 7.2.2.2 Can also use Diluent 2 (VWR Cat#45001-012)
  - 7.2.3 BD FACS Clean
    - 7.2.3.1 BD Cat# 340345
  - 7.2.4 Germicidal Bleach
    - 7.2.4.1 VWR Cat# 89501-620
  - 7.2.5 Coulter Clenz Cleaning Agent, 5L, Beckman Coulter ref# 8546930
    - 7.2.5.1 VWR Cat# BK8546930
  - 7.2.6 Contrad 70, 5L, Decon Laboratories, Inc. Cat# 1003
    - 7.2.6.1 VWR Cat# 42000-000
- 7.3 Consumables
  - 7.3.1 Disposable Gloves, Black Nitrile Exam Gloves
    - 7.3.1.1 Small, VWR Cat# 10860-870
    - 7.3.1.2 Medium, VWR Cat# 10860-852
    - 7.3.1.3 Large, VWR Cat# 10860-834
  - 7.3.2 Falcon 5ml Polystyrene Round-Bottom Tubes, no caps, ref. 352052
    - 7.3.2.1 VWR Cat# 60819-138
  - 7.3.3 Falcon 5ml Polystyrene Round-Bottom Tubes, with caps, ref. 352054
    - 7.3.3.1 VWR Cat# 60819-310
  - 7.3.4 Falcon, 5ml Polystyrene Round-Bottom Tubes with Cell Strainer Cap, 35µn, ref. 352235
    - 7.3.4.1 VWR Cat# 21008-948
  - 7.3.5 VWR Disposable Transfer Pipettes
    - 7.3.5.1 VWR Cat# 414004-005
  - 7.3.6 VWR 1000µl Pipet Tips
    - 7.3.6.1 VWR Cat# 83007-378
  - 7.3.7 VWR 200µl Pipet Tips
    - 7.3.7.1 VWR Cat# 53503-602

8. OPERATION

- 8.1 Startup of BD LSRFortessa-HTS
  - 8.1.1 Make sure that a tube with 1ml of deionized water is on the SIP and the tube support arm is centered.
  - 8.1.2 Power ON the cytometer. System power button located on the right side panel of the instrument. This will turn on the power to the cytometer and lasers. Allow 30 minutes for the lasers to warm up and stabilize.

8.1.3 Sign into the kiosk to “Start” your reservation and power ON the workstation monitors (see SOP# GA001: iLab – Registration, Scheduling Reservations and Kiosk).

8.1.4 Power ON the workstation. Windows requires a user login with case sensitive password to startup (*user: XXXXXX, password: XXXXXX*). Allow workstation to complete all startup processes.

8.1.5 Start Coherent Connection and verify that the lasers are on and ready.

8.1.6 Start BD FACSDiva Software and login to your account (Principal Investigator/Lab name).

8.1.7 Allow BD FACSDiva Software to connect to cytometer.

8.1.8 Make sure all necessary windows are displayed.

8.1.8.1 Browser

8.1.8.2 Cytometer

8.1.8.3 Inspector

8.1.8.4 Acquisition Dashboard

8.1.8.5 Global Worksheet

8.1.9 Check fluid levels. Make sure cytometer is in STNDBY mode.

8.1.9.1 Waste container: empty then add 200ml of bleach.

8.1.9.2 Sheath tank: replace with FACSFlow box or Diluent 2 cube.

8.2 Sample Acquisition and Data Recording

8.2.1 Sample tube specifications

8.2.1.1 Must use 12x75mm (5ml) polystyrene tubes.

8.2.1.2 Cells should be in 0.5ml suspension (not to exceed 1ml).

8.2.1.3 Must filter cells using a 35-45μn nylon mesh prior to acquisition.

8.2.2 Acquisition and recording procedures

8.2.2.1 Set cytometer to RUN and LO on the cytometer fluid control panel. The LO setting will allow you more time to adjust settings, however, if you have a small number of cells you will need to run your samples either on MED or HI.

8.2.2.2 Move tube support arm to the side and remove water tube by pulling straight down, do not twist and pull to remove tube as this loosens the SIP.

8.2.2.3 Install sample tube onto the SIP, ensuring proper seating on the BAL seal.

8.2.2.4 Move the tube support arm to the centered position and acquire samples. Adjust settings and record data. Do not allow sample tubes to run dry as this will introduce air into the sample line and cause the instrument to malfunction.

8.2.2.5 When recording is complete, remove sample tube from SIP. If you need extra time between samples, install a water tube onto the SIP, center the tube support arm, and place instrument on STNDBY. NEVER allow tube support arm to be in the centered position without a tube of water.

8.2.2.6 Proceed to next tube and continue acquiring your data.

8.2.2.7 Clean instrument when sample acquisition is complete. Run FACS Clean then DI water for 3 minutes each.

8.2.2.8 Save data files to the Desktop for transfer to external storage systems.

8.2.2.9 If you are the last user, proceed to shutdown procedures. If not the last user, logout of FACSDiva Software, then logout of the kiosk, leaving the instrument ready for the next user.

8.3 Using High Throughput Sampler (HTS)

8.3.1 Preparing the cytometer for the HTS

8.3.1.1 Manually change instrument from “tube” to “plate” using switch located on the right side panel of the instrument.

8.3.1.2 Power ON the HTS unit. Power switch located on the back of the unit.

8.3.1.3 Acquisition of samples using the HTS requires more sheath fluid than acquisition using tubes, therefore, ensure there is enough sheath fluid to prime the HTS unit, run your samples, and clean the unit after acquisition. This may require changing out the sheath fluid with a new, full cube and emptying the waste.

8.3.2 Preparing HTS for acquisition of samples

8.3.2.1 Move support arm to the side and remove water tube from the SIP.

8.3.2.2 Remove the outer sleeve of the sample injection tube and replace with the sample injection tube protector.

8.3.2.3 Connect the sample coupler to the sample injection tube.

8.3.2.4 Check that waste and sheath tubing and communication cables are securely connected.

8.3.2.5 Open BD FACSDiva Software, set cytometer to RUN and LO on the cytometry fluid control and prime the HTS unit by clicking HTS > Prime. Repeat priming 2-3 times. This ensures that sheath fluid is running through the unit and all the bubbles have been removed. HTS unit is now ready to acquire samples.

8.3.3 Acquisition and recording procedures using the HTS

8.3.3.1 It is strongly recommend that experiment setup (adjustment of voltages and compensation) be done using tubes prior to preparing HTS unit for acquisition of samples.

8.3.3.2 HTS unit is capable of processing 96 well plates (flat bottom, "U" bottom, or conical bottom).

8.3.3.3 Highlight wells for acquisition and set parameters in plate window. Select random wells and verify settings.

8.3.3.4 Acquire data by highlighting sample wells. Then click on the "Run Well(s)" button on the acquisition dashboard. The sample probe will begin to move, and the instrument will begin to process the samples in the plate using the previously determined parameters. Data is automatically recorded as the samples are processed.

8.3.4 Cleaning HTS unit after acquisition of samples

8.3.4.1 After acquisition of samples, prepare a 96 well plate with 200µl of FACSClean in wells A1 – A4 and 200µl of deionized water in wells B1 – B4. Place plate on the plate holder of the HTS unit.

8.3.4.2 Click on HTS > Clean. Select the "Daily Clean" template. Click OK.

8.3.4.3 When "Daily Clean" is complete, disconnect sheath line from unit and connect a 50ml syringe with deionized water. Click HTS > Prime and repeat 3-4 times. This removes all the sheath fluid in the HTS unit to prevent crystallization of salt from the sheath fluid.

8.3.4.4 Disconnect the 50ml syringe and reconnect sheath line. Disconnect the sample coupler from the sample injection tube, remove the sample injection tube protector and replace with outer sleeve of the sample injection tube.

8.3.4.5 Place a tube with 1ml of water on the SIP and set the cytometer on STNDBY.

8.3.4.6 Manually change the instrument from "plate" to "tube" and power OFF HTS unit.

8.4 Shutdown of BD LSRFortessa-HTS

8.4.1 Completed at the end of the day by either the last scheduled user or Flow Cytometry Shared Resource staff.

8.4.1.1 Close BD FACSDiva Software. Click File > Quit.

8.4.1.2 Close Coherent Connection. Click File > Exit.

8.4.1.3 Shutdown workstation.

8.4.1.4 Sign into the kiosk to "Finish" your reservation and power off the workstation monitors (see SOP# GA001: iLab – Registration, Scheduling Reservations and Kiosk).

8.4.1.4 Turn off the power to the cytometer.

8.5 BD LSRFortessa-HTS Data Storage Policy

8.5.1 Users of the UM SCCC Flow Cytometry Shared Resource are responsible for saving and exporting their data.

8.5.1.1 Data files should first be temporarily saved onto the workstation Desktop then copied to the facility's server (S: drive), the users laboratory shared drive or Box (a secure cloud-based storage solution approved and recommended by the University of Miami, Miller School of Medicine). Use of external drives such as USB Flash drives are not allowed on the instrument workstations.

8.5.2 Every month, users are responsible for deleting all experimental data located in their Browser within the BD FACSDiva Software.

8.5.2.1 Experiments older than 2 months will be deleted by the Flow Cytometry Shared Resource staff.

8.5.2.2 If experimental templates are created, users must label them appropriately and request permission to keep them in their Browser for an extended period of time.

9. CALIBRATION

9.1 BD Cytometer Setup and Tracking Beads

9.1.1 The UM SCCC Flow Cytometry Shared Resource will perform a baseline of CS&T with every new lot number of CS&T beads.

9.1.2 Monday thru Friday, the UM SCCC Flow Cytometry Shared Resource will perform a daily CS&T performance check.

9.1.2.1 Exception #1: If the instrument is offline (general).

9.1.2.2 Exception #2: If the instrument is offline and awaiting repairs.

9.1.2.3 Exception #3: If the instrument is offline due to a scheduled PM (service engineer would then complete CS&T).

9.1.2.4 Exception #4: If the instrument is heavily booked and core staff is unable to perform the daily CS&T performance check.

9.1.3 If they require it, trained users can perform a CS&T performance check during weekend hours.

9.1.4 Should CS&T not pass specifications, then maintenance will be performed (to remove clog or air bubbles), if CS&T should continue to fail, See 10.5 (service call)

10. MAINTENANCE

10.1 Daily Maintenance Procedures

10.1.1 Perform daily startup and shutdown procedures.

10.1.1.1 Purge sheath filter when a sheath fluid cube has been replaced.

10.1.2 Check fluid levels. Clean or replace any components of the fluidics system.
10.1.3 Clean cytometer with BD FACS Clean and DI water. Alternatively, can use Coulter Clenz or Contrad 70.
10.1.3.1 Set cytometer to RUN and HI on the cytometer fluid control panel.
10.1.3.2 Install a tube with 3ml of BD FACS Clean solution.
10.1.3.3 Set the tube support arm to the side and let it run for 1 minute. Then place the tube support arm in the center position and allow to run for 5 minutes. Remove tube.
10.1.3.4 Install a tube with 3ml of DI water.
10.1.3.5 Set the tube support arm to the side and let it run for 1 minute. Then place the tube support arm in the center position and allow to run for 5 minutes. Remove tube.
10.1.3.6 Set cytometer to STNDBY on the cytometer fluid control and install a new tube with clean DI water on the SIP.
10.1.4 Wipe down the SIP and tube support arm with a wet kim wipe to prevent saline deposit buildup.
10.2 Bi-Monthly Maintenance Procedures
10.2.1 Perform system flush. This procedure removes debris and contaminants from the sheath tubing, waste tubing, and flow cell.
10.2.1.1 Turn off the FACSFlow Supply Cart.
10.2.1.2 Remove the sheath filter and connect the two fluid lines.
10.2.1.3 Empty the pressurized sheath tank and rinse it with DI water.
10.2.1.4 Fill the pressurized sheath tank with at least 1L of 10% bleach and, if needed, empty the waste container. Re-connect both containers to cytometer. Alternatively, you can have a dedicated sheath tank for 10% bleach.
10.2.1.5 Remove DI water tube from SIP and prime twice.
10.2.1.6 Install a tube with 3ml of 10% bleach on the SIP and run on HI for 45 minutes.
10.2.1.7 Repeat steps 10.2.1.3 to 10.2.1.6 with DI water. Make sure to wash the tank with DI water 3 times to remove all of the bleach. Alternatively, you can have a dedicated sheath tank for DI water.
10.2.1.8 Replace the sheath filter and refill the pressurized sheath tank with sheath fluid. Repeat steps 10.2.1.3 to 10.2.1.6 with sheath fluid.
10.3 Preventative Maintenance (PM)
10.3.1 Preventative maintenance is conducted by a BD service engineer and scheduled every 6 months.

10.3.1.1 BD service engineer keeps record of this and will contact us for scheduling date and time.

10.4 Unscheduled Maintenance
  - 10.4.1 Changing the sheath filter. Usually done during PM, however, can be changed when necessary.
  - 10.4.2 Changing the BAL seal. BAL seal ensures proper tube pressurization, therefore, should be replaced when needed if worn, cracked, or proper seal is not formed.
  - 10.4.3 Changing the sample tube O-ring. Replace when drops form at the end of the SIP while the vacuum is operating.
  - 10.4.4 Keeping external surfaces clean and free from salt buildup.
  - 10.4.5 If cytometer will be taken offline for long-term storage, decontaminate the fluidics system with BD FACS Clean.

10.5 Contacting BD for Servicing and Repairs
  - 10.5.1 Call BD Service for servicing and repairs at 1-877-232-8995, option #2.
  - 10.5.2 Have ready the instrument serial number and a description of the problem.
  - 10.5.3 Record issue and reference number in service call book. Record date of servicing and a description of the resolution.